METHODS FOR DETERMINING SQUALENE SYNTHASE ACTIVITY

ABSTRACT

The cloning of a truncated Arabidopsis gene expressing squalene synthase, as well as the expression and purification of the squalene synthase, are described. Also described herein is a fluorescent assay using squalene synthase that is amenable to high-throughout use, particularly for studying the regulation of isoprenoid synthesis and identifying squalene synthase inhibitors and promoters. As the formation of squalene is stoichiometric with the depletion of NADPH, the activity of squalene synthase can be evaluated by following the NADPH concentration over time. Squalene synthase activity is determined by combining FPP, NADPH, squalene synthase and a magnesium ion cofactor to form a reaction mixture under conditions suitable for squalene formation, optionally in the presence of a compound being analyzed for its ability to inhibit or promote squalene synthase. The concentration of NADPH over time is determined by subjecting the reaction mixture to UV light and detecting fluorescent light emission.